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Characterization of the *snowy cotyledon 1* mutant of *Arabidopsis thaliana*: the impact of chloroplast elongation factor G on chloroplast development and plant vitality

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Abstract

During seedling development chloroplast formation marks the transition from heterotrophic to autotrophic growth. The development and activity of chloroplasts may differ in cotyledons that initially serve as a storage organ and true leaves whose primary function is photosynthesis. A genetic screen was used for the identification of genes that affect selectively chloroplast function in cotyledons of *Arabidopsis thaliana*. Several mutants exhibiting pale cotyledons and green true leaves were isolated and dubbed *snowy cotyledon* (*sco*). One of the mutants, *sco1*, was characterized in more detail. The mutated gene was identified using map-based cloning. The mutant contains a point mutation in a gene encoding the chloroplast elongation factor G, leading to an amino acid exchange within the predicted 70S ribosome-binding domain. The mutation results in a delay in the onset of germination. At this early developmental stage embryos still contain undifferentiated proplastids, whose proper function seems necessary for seed germination. In light-grown *sco1* seedlings the greening of cotyledons is severely impaired, whereas the following true leaves develop normally as in wild-type plants. Despite this apparent similarity of chloroplast development in true leaves of mutant and wild-type plants various aspects of mature plant development are also affected by the *sco1* mutation such as the onset of flowering, the growth rate, and seed production. The onset of senescence in the mutant and the wild-type plants occurs, however, at the same time, suggesting that in the mutant this particular developmental step does not seem to suffer from reduced protein translation efficiency in chloroplasts.

Introduction

Chloroplasts have evolved from prokaryotic progenitors that initially invaded eukaryotic host cells and lived as endosymbionts that gradually translocated most of their genes during evolution into the nucleus of the host cells. Chloroplasts of higher plants have retained less than 100 genes, whereas more than 2000 genes encoding plastid proteins form part of the nuclear genome (Ahlert *et al.*, 2003). Thus, chloroplast development is based on a highly coordinated interaction between nucleus and plastids that includes also the import of numerous nuclear-encoded plastid proteins into the plastid compartment and the assembly of mixed membrane complexes, consisting of nuclear and plastid DNA-encoded proteins.

Various genetic approaches have been used to disrupt this interplay between plastid and nucleus and to determine the importance of different chloroplast activities for the overall performance of plants throughout their life cycle. Pigment-

deficient plants such as *albino* or *xantha* mutants do not survive beyond the seedling stage and have to be rescued as heterozygous lines (Runge *et al*., 1995; Bisanz *et al*., 2003). In other mutants pigment deficiencies are restricted either to cotyledons or to true leaves and in contrast to the *albino* and *xantha* mutants these plants continue to grow, albeit often at a reduced rate. The development of plastids in cotyledons differs in several aspects from that in true leaves. As long as seedlings grow submerged in the soil they live heterotrophically by using nutrients stored mainly in cotyledon cells. At this initial stage of development etioplasts develop from proplastids that are devoid of chlorophyll, but instead accumulate minor amounts of protochlorophyllide, the immediate precursor of chlorophyll, that upon illumination is photo-reduced to chlorophyll. Once the seedling emerges from the soil and reaches the light it rapidly changes from heterotrophic to autotrophic growth by transforming etioplasts into chloroplasts that soon after the beginning of illumination enable the plant to photosynthesize. In contrast to chloroplast formation in cotyledons, in true leaves chloroplasts develop directly from proplastids. This difference in chloroplast development is also reflected in different import specificities in cotyledons and true leaves for the NADPH-protochlorophyllide oxidoreductase A (PORA) that catalyzes the light-dependent reduction of protochlorophyllide to chlorophyllide (Kim and Apel, 2004). In plastids of cotyledons the import of this nuclear encoded protein requires protochlorophyllide, whereas in true leaves the import occurs independently of protochlorophyllide. Differences in the regulation of plastid development of cotyledons and true leaves were also revealed more indirectly by the identification of mutants with pigment deficiencies that were either confined to cotyledons or to true leaves. In the variegated mutants *var2* and *var3* of *Arabidopsis* cotyledons developed normally but true leaves contained pigment-deficient white areas (Chen *et al*., 2000; Naested *et al*., 2004). A reverse distribution of pigment deficiencies was found in the *white cotyledon* (*wco*) mutant (Yamamoto *et al*., 2000) and in sigma 2 antisense plants (Privat *et al*., 2003) with chlorophyll-deficient cotyledons but green true leaves.

In the present work we have isolated a new set of mutants that were dubbed *snowy cotyledon* (*sco*). One of these mutants, *sco1*, was characterized in greater details. Chlorophyll deficiency in this mutant was restricted to the cotyledons, whereas true leaves contained almost normal amounts of chlorophyll and in this respect were indistinguishable from wild-type leaves. Despite these similarities mutant and wild-type plants differed in several other ways, indicating that the slight disturbance in chloroplast development that is obvious only at the cotyledon stage may have a long-lasting effect at different stages of plant development ranging from seed germination to flower formation. The *SCO1* gene was identified by map based cloning and offered a clue as to how the mutation could result in pigment-deficient cotyledons.

Materials and methods

Plant material and identification of the sco1 *mutation*

Seeds of the *Arabidospis thaliana* ecotype *Landsberg erecta* were chemically mutagenized with ethyl-methylsulfonate (EMS) and the M2 progeny screened for seedlings with pale cotyledons and normal looking true leaves. One of the isolated mutants was the *sco1* mutant. To identify the mutated gene, we crossed the homozygous mutant with Columbia wild-type plants. Homozygous lines with a *sco1* phenotype in the F2 generation were used for further mapping of the mutated gene using specific primers for SSLP markers. Mapping primers have been designed using information of the Monsanto database from "The Arabidopsis Information Resource" (TAIR, http://www.arabidopsis.org). Plants were grown under long-day conditions (16 h light, 8 h dark) at 21 °C. Surface-sterilized seeds were sown on Murashige–Skoog (MS) media, in most cases without sucrose. Sucrose (0.5%) containing media were only used where indicated.

Cloning of the SCO1 *gene*

For the complementation analysis gene specific primers containing *Nco*I restriction sites were used for the amplification of the genomic wild-type fragment. To investigate the localiza-

tion of the protein cDNA of SCO1 without the terminal STOP codon was obtained by PCR from reverse-transcribed total mRNA. We subsequently cloned the amplified and restricted fragments into the plant binary vector pCAMBIA 1302, which contains the coding sequence for the reporter green fluorescent protein (Hajdukiewicz *et al*., 1994). Plants were transformed with the *Agrobacterium tumefasciens* C58 strain using the floral dip method (Clough and Bent, 1998). Transgenic plants were isolated after growth on selective MS media containing hygromycin (20 mg/l). Transgenic mutant plants were identified as complemented if the cotyledons appeared green like wild-type cotyledons. Their identity was confirmed by PCR with gene- and vector-specific primers. GFP analysis has been performed under a confocal laser-scanning microscope (Leica Microsystems, Heidelberg Germany) with protoplasts extracted from transgenic wild-type plants. The mRNA analysis has been performed by PCR with gene-specific primers on reverse-transcribed, DNase-treated total mRNA extracted from plant material harvested at the indicated time points.

Pigment analysis

For pigment analysis of seedlings 30 mg fresh weight per sample from 3, 7, and 14-day-old seedlings were harvested. Leaf material of 14-day-old seedlings was divided into cotyledons and first true leaves. For the chlorophyll measurement of the fifth rosette leaf from mature plants leaves were marked with a red thread and harvested at different ages. The chlorophyll content was expressed per g fresh weight and each measurement was done in triplicate. The plant material was immediately frozen in liquid nitrogen. The leaf material was ground in 750 μl acetone with barium carbonate and centrifuged twice to remove cell fragments. The supernatant was used for further analysis and pigments were separated by HPLC as described (Gutensohn *et al*., 2004). The pigment content of wild-type seedlings was taken as 100% and the chlorophyll content of mutant seedlings expressed as % of wild type. The pigment content of mature leaves was expressed per fresh weight of each sample.

Protein analysis

Seedlings were harvested at the indicated time points and proteins extracted as described (Kim and Apel, 2004). For western analysis 25 μg of protein per sample were loaded onto an SDS gel, and separated by electrophoresis. LHCP, D1, and CP22 proteins were immunologically detected using monospecific antisera obtained from Agri-Sera (Sweden). As a loading control immunodetection of the cytosolic PDX was used (antisera kindly provided by Theresa B. Fitzpatrick, ETH Zurich). Subunits of the rubisco holoenzyme were detected using antibodies raised against the holoenzyme of barley (kindly provided by Matthias Schmidt, University Frankfurt). The immunoreactive proteins were visualized on a BioMax light film (Kodak) following hybridization of the blot with the Immuno-Star^{TM} HRP (BIORAD). The analysis was repeated three times.

Growth and vitality measurements

Growth rate of plants was determined by measuring the length of the bolting stem of several plants of wild type and the *sco1* mutant. The fresh weight of the above-ground plant material was taken from the same plant from which the fifth leaf was used for pigment analysis. The vitality of plants was estimated based on the amounts of seeds produced per plant.

Results

Isolation and identification of the sco1 *mutant*

During early development seedlings undergo a transition from heterotrophic to autotrophic growth. This transition to autotrophic growth requires a light-induced rapid transformation of etioplasts to chloroplasts that seems to be restricted to cotyledons and normally does not occur in true leaves. The aim of our work was to identify mutants that exhibit a cotyledon-specific disturbance of chloroplast development with pale cotyledons and green true leaves. Seeds of *Arabidopsis thaliana* were mutagenized chemically with ethylmethylsulfonate (EMS) and were grown on soil (M1 plants). Approximately 25.000 M2 seedlings

derived from the M1 plants were grown on MS agar plates under continuous light and mutants with pale cotyledons but green true leaves were selected. Several of such mutants were identified and dubbed *snowy cotyledon*. Allelism tests of these mutants revealed two different loci (*sco1* and *sco2*). One of the mutants, *snowy cotyledon 1* (*sco1*), was characterized in more detail. Cotyledons of 3-day-old mutant seedlings were white (Figure 1A). After 14 days the cotyledons were still pale, whereas the true leaves were green (Figure 1B). During this developmental stage the size of the leaves of mutants were smaller than that of wild-type leaves. Backcrossing of homozygous *sco1* mutants with wild-type plants confirmed that the phenotype of the mutant was due to one recessive mutation. The mutated gene was identified by map-based cloning. The mutated gene could be mapped to the lower arm of chromosome I, approximately 2.8 cM distal to the marker nga280 (Figure 2A). The mutation was localized on the 40 kb region of the BAC clone F23N19 (Acc.no. AC007190). Due to the pigment-deficient cotyledons of *sco1* seedlings, we expected the mutation to affect a gene encoding a plastid-specific protein. Within the 40 kb region of the BAC clone three genes encoding proteins with predicted chloroplast-import signals were found. They were amplified by PCR and subsequently sequenced. In one of these genes (At1g62750) a point mutation with a G-to-A mutation was detected. This nucleotide exchange caused an amino acid change from glycine to arginine at position 132 (G132R). The gene At1g62750 encodes a predicted chloroplast-localized elongation factor G later named AtSCO1/cpEF-G. The open reading frame consists of four exons of an mRNA transcript of 2352 bp and a protein of 783 amino acids. A BLAST search of the sequence database revealed no other gene encoding a chloroplast-localized EF-G-like protein in the *Arabidopsis* genome but two mitochondrial EF-G genes. Transformation of homozygous *sco1* mutant plants with the genomic fragment of At1g62750 of wild-type complemented the mutant phenotype in that cotyledons of the complemented mutant accumulated chlorophyll similar to wild-type but in contrast to the parental *sco1* mutant seedling (Figure 2D). The complementation was not complete as indicated by the difference of seedling size of several complemented mutant lines and the wild-type. The incomplete complementation may be due to the fact that within the complemented mutant seedlings the mutated *sco1*/*cpEF-G* factor is still expressed and may reduce the activity of the wild-type copy of SCO1/cpEF-G by competing for a common target.

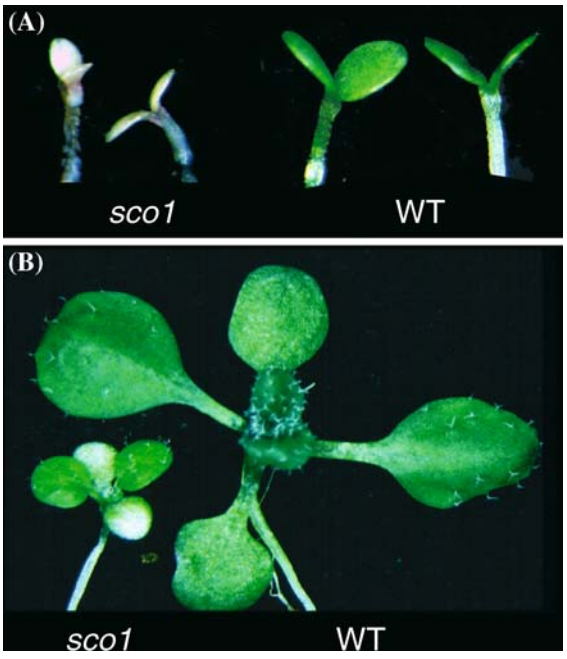

Figure 1. A comparison of 3-day-old (A) and 14-day-old (B) seedlings of *sco1* mutant and wild-type plants of *Arabidopsis thaliana*.

The encoded protein consists of the five domains conserved in EF-G proteins, with two GTP-binding sites in the first domain, and an additional transit peptide at the N-terminus (Figure 2B). The mutation in At1g62750 affects the protein sequence in front of the second GTP-binding effector domain. Comparison of this region with other EF-G protein sequences from plants like *Glycine max* or bacteria (*E. coli* and *A. tumefasciens*) revealed that the mutated glycine is highly conserved (Figure 2C). Stable transformation of wild-type plants with SCO1 cDNA fused to the green fluorescent reporter gene revealed the colocalization of the green fluorescence of the reporter protein with the autofluorescence of chlorophyll, indicating that the SCO1 protein acts within chloroplasts (Figure 2E).

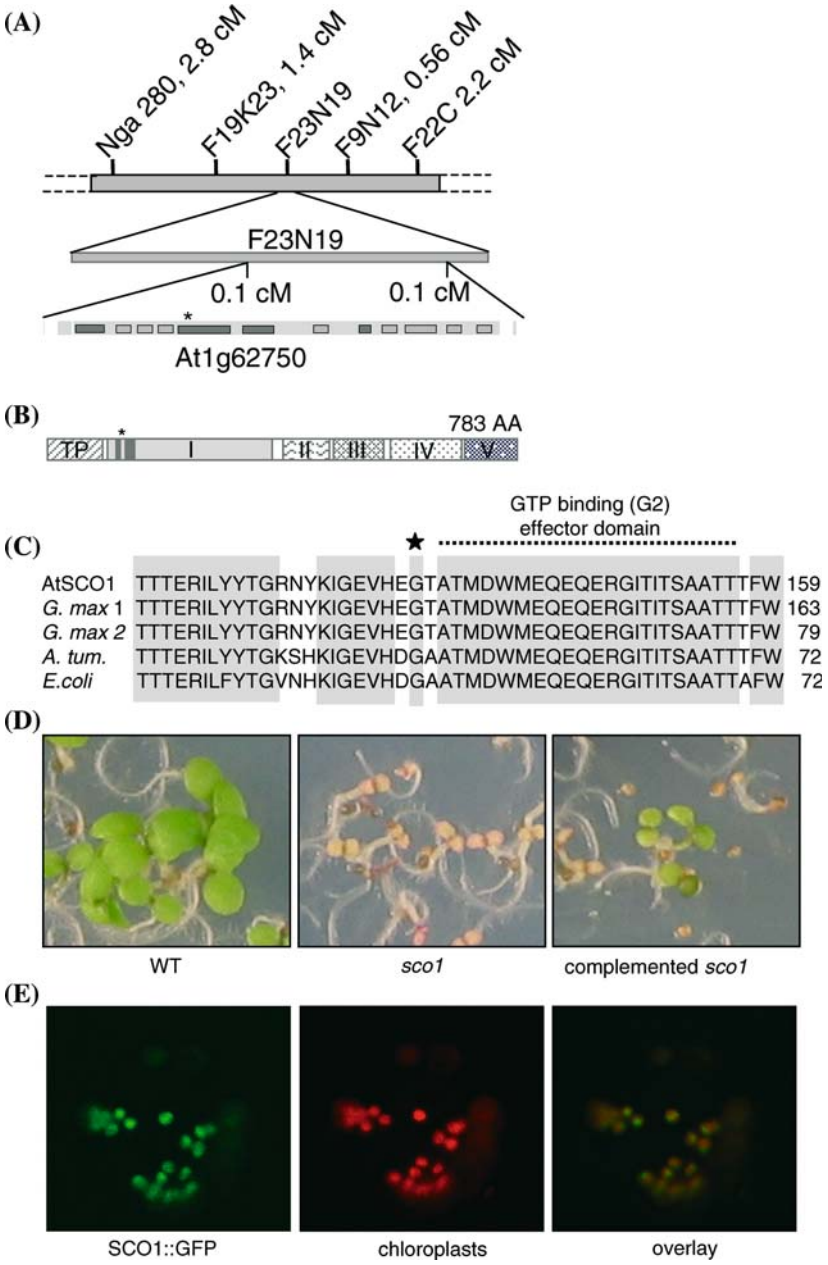

Figure 2. Identification of the *SCO1* gene. (A) Genetic and physical map of the DNA region on chromosome I of *A. thaliana* and the BAC clone F23N19 that contain the *SCO1* gene. Predicted genes on the cloned BAC fragment are indicated by boxes, those predicted to encode plastid proteins are shown in dark grey. One of these genes, AT1g62750, contains a point mutation as indicated by the asterisk. (B) Schematic diagram of the deduced amino acid sequence of cpEF-G containing domains I to V and the transit peptide (TP) at the N-terminus of the protein. The dark grey regions in domain I represent the two GTP-binding sites of AtSCO1/cpEF-G. The asterisk marks the position of the mutation. (C) Comparison of the deduced amino acid sequence of the GTP-binding effector domain of AtSCO1/cpEF-G (At1g62750) with that of other known sequences of EF-G proteins from *Glycine max* 1 (S35701), *G. max* 2 (CAA50573), *Agrobacterium tumefaciens* (NP_354925), and *E. coli* (NP_312218). Conserved amino acids are highlighted by grey boxes. The stretch of the GTP-binding effector domain is marked by the dashed line. The site of the amino acid exchange in the protein of the *sco1* mutant is marked with an asterisk. (D) Complementation of the *sco1* mutant with the wild-type fragment of At1g62750. (E) Localization of the SCO1::GFP fusion protein in chloroplasts of protoplasts isolated from stable transformed wild-type plants.

Characterization of the sco1 *mutant*

The *sco1* mutant was isolated because of its pigment deficiency that seemed to be restricted to cotyledons. The 3-day-old *sco1* mutant seedlings were indistinguishable from *albino* mutants (Figure 3A). However, in 1 week old seedlings a slight greening of the cotyledons began and in 14-day-old seedlings the cotyledons had turned green except for a white tip. The true leaves of the 14-day-old seedlings looked green and their color was indistinguishable from that of wild-type leaves. The initial growth of mutant seedlings was delayed when compared to that of the wild-type seedlings of the same age. At this stage the delay in seedling development and pigment accumulation could not be overcome by growing the mutant on sucrose-containing media (Figure 3B).

The extent of pigment deficiency and the slow greening of *sco1* seedlings was quantified by comparing pigment contents of mutant seedlings (30 mg fresh weight per sample) with those of wild-type seedlings at different time points of seedling development. Only about 20% of chlorophyll *a* and *b* could be detected in 3-day-old seedlings of *sco1* (Figure 4A). After one week the chlorophyll level in mutant seedlings reached about 50% of wild type and increased to 70% in 2 week-old seedlings. In mature leaves of 4 week-old plants about 80% chlorophyll of the wild-type level was detected, indicating that the true leaves of the mutant plant still have a slightly reduced pigment content and that the observed phenotype was not restricted to cotyledons. The same differences in pigment concentration could also be observed for lutein and β-carotenoid (data not shown).

The impairment of a factor necessary for mRNA translation in chloroplasts raises the question whether the synthesis of plastid proteins is visibly affected in the *sco1* mutant. Total protein of *sco1* and wild-type seedlings at different stages of development was isolated, 25 μg protein per sample loaded on an SDS page, and a western analysis performed using specific antibodies against both subunits of the rubisco holoenzyme. Not only the pigment accumulation but also the synthesis of the two rubisco subunit polypeptides was delayed in the *sco1* seedling (Figure 4B). In 3-day-old *sco1* seedlings almost no subunit of

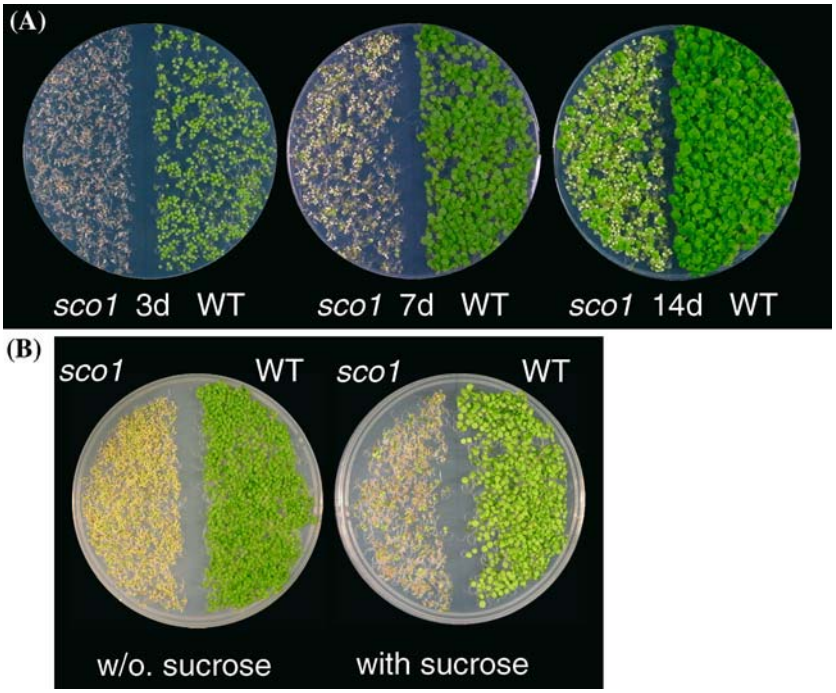

Figure 3. Comparison of seedlings development of the *sco1* mutant and wild type (WT) grown for different lengths of time (A) with or without sucrose in the medium (B). Seedlings in (A) were grown for 3, 7 and 14 days under continuous light on MS agar. Seedlings in (B) were grown for 3 days under continuous light on MS agar with or without (w/o) 0.5% sucrose.

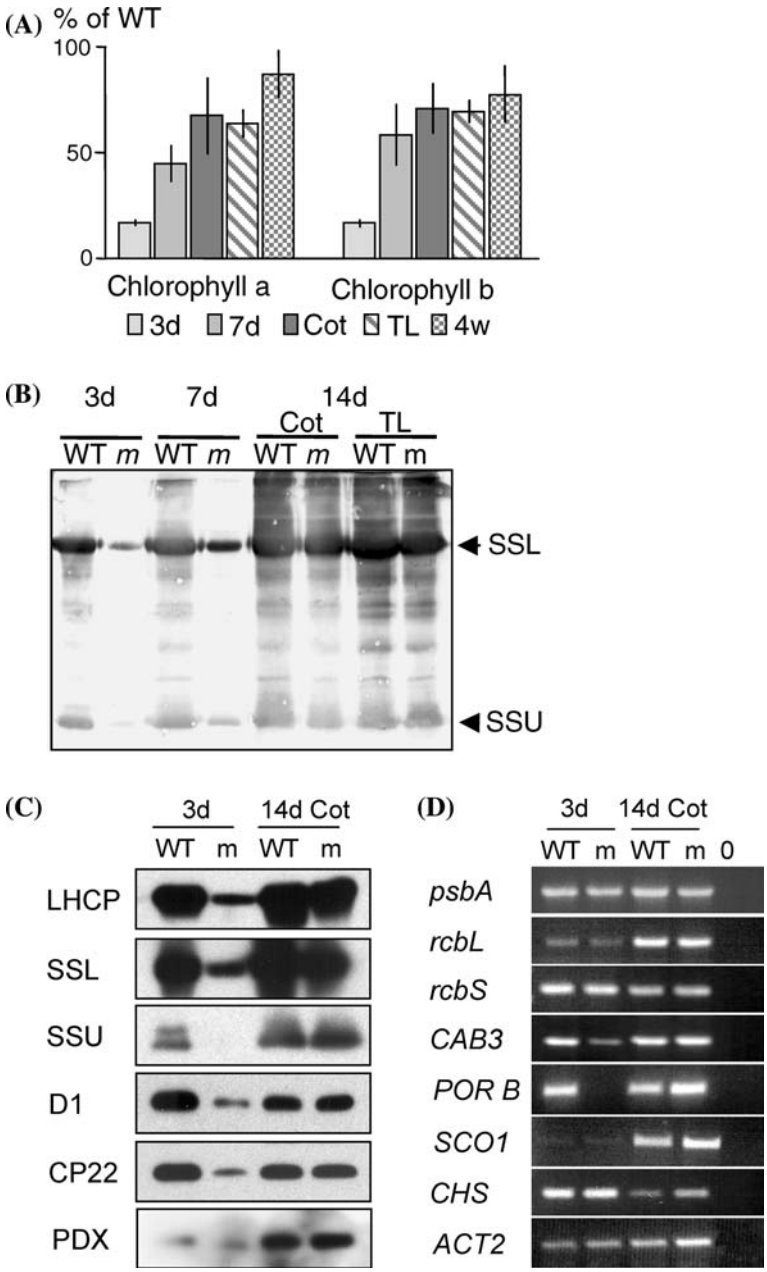

Figure 4. Changes in chlorophyll (A) and ribulose−1,5-bisphosphate-carboxylase protein (Rubisco) (B) content of the *sco1* mutant. In (A) chlorophyll *a* and *b* was determined in 3 and 7-day-old seedlings and in cotyledons (Cot) and true leaves (TL) of 14-day-old seedlings, and in leaves of 4 week-old plants (4w). The chlorophyll levels in the mutant (m) are expressed relative to those of wild type (WT) control plants of the same age that were set as 100%. In (B) equal amounts of total protein of 3 (3d), 7 (7d) and 14-day-old (14d) seedlings were separated electrophoretically and transferred onto membranes. The small (SSU) and large (LSU) subunits of rubisco were detected immunologically using an antiserum raised against the holoenzyme of Rubisco. In the case of 3 and 7-day-old seedlings total protein extracts of seedlings were analyzed, whereas in the case of 14-day-old seedlings cotyledons and true leaves were cut from the seedlings and analyzed separately (Cot, TL). (C) Immunodetection with monospecific antisera of the chloroplast proteins LHCP, LSU, SSU, D1, and CP22 as well as the cytosolic protein PDX as a loading control on proteins extracted from 3 and 14-day-old wild-type and mutant cotyledons. (D) RT-PCR with cDNA-specific primers on reverse transcribed, DNase treated mRNA extracted from 3-day- and 14-day-old cotyledons.

rubisco was detectable, but the amount of both subunits gradually increased during the following days and finally after 14 days reached wild-type levels in cotyledons and true leaves. The relative content of other plastid proteins such as LHCP, D1, and CP22 in 3-day- and 14-day-old cotyledons of wild type and mutant seedlings were determined immunologically (Figure 4C). In all cases protein levels in cotyledons of 3-day-old seedlings were drastically reduced in the mutant, whereas in 14-day-old seedlings they reached similar levels as in wild type. If the difference in protein accumulation during the early stage of development was due to the impairment of the plastid-specific elongation factor G the mutation should affect the translation but not the actual concentrations of the plastid DNA transcripts. The mRNA levels were tested by RT-PCR using cDNA-specific primers for the two plastid DNA-specific transcripts *psbA* and *rcbS* and transcripts for the nuclear encoded plastid proteins SSU, LHCP, and PORB. Also transcripts for *SCO1* and the cytosolic chalcone synthase (CHS) were analyzed. *ACT2*-transcripts were used for a loading control. There were no detectable differences between chloroplast DNA-specific mRNA, rcbS, and chalcone synthase transcript levels of wild-type and mutant seedlings (Figure 4D). However, transcripts for the two nuclear encoded plastid proteins LHCP and PORB were strongly reduced in 3-day- but not in 14-day-old seedlings. These results suggest that the translation but not the transcription of plastid-specific mRNAs is suppressed in the mutant and that this disturbance contributes to the delay of pigment accumulation in *sco1* mutant seedlings.

The mutant plants are delayed in germination

The defect in the *sco1* mutant does not only affect pigment accumulation in greening seedlings but also the germination of seeds. Seeds were plated on MS agar and kept for 24 h at 4 °C in the dark before transferring them to 21 °C and exposing them to light for 1 h. Half of the seeds were returned to the dark, whereas the second half was kept under continuous light. Under both conditions seed germination of the *sco1* mutant was delayed (Figure 5A). Since in dark-grown seedlings chlorophyll synthesis does not occur the delay of *sco1* seed germination cannot be attributed to a deficiency in photosynthetic activity of the *sco1* mutant but must be due to the disturbance of other activities of plastids that are required for germination and seem to depend on protein synthesis in the plastid compartment.

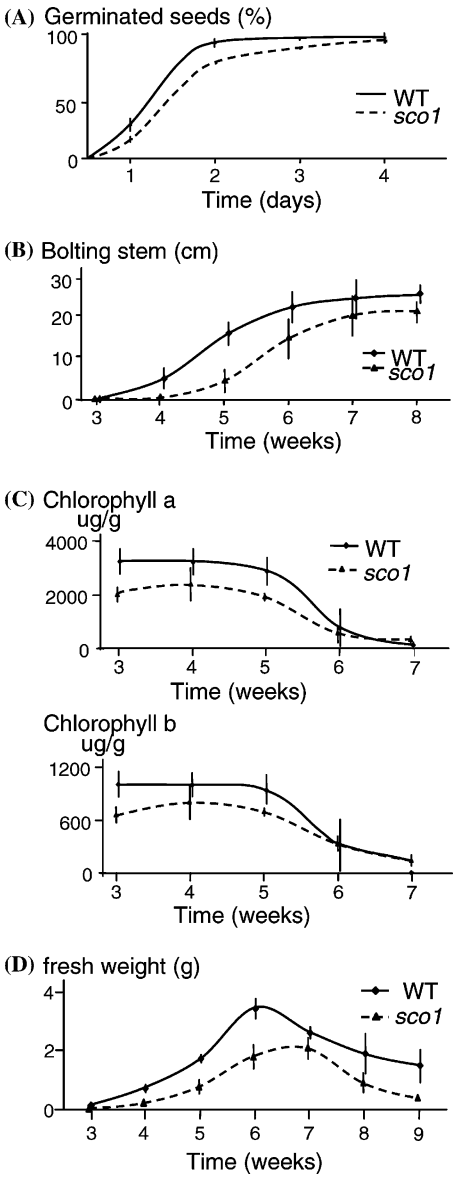

Figure 5. Seed germination (A) and growth of mature plants (B) in wild type (WT) and *sco1* mutant plants of *Arabidopsis thaliana*. (C) Changes in the amounts of chlorophyll *a* and *b* of the fifth rosette leave during senescence in wild type (WT) and *sco1* mutant plants. The chlorophyll content is expressed per gram fresh weight of leaf material. (D) Changes in fresh weight of wild type and mutant plants during flowering and senescence.

Comparison of adult plants

The delay in development was not only evident during germination but also in mature plants. Whereas the bolting of wild-type plants started after 4 weeks, the onset of bolting in mutant plants was delayed by 1 week (Figure 5B). The maximum length of the principal inflorescence stem of the mutant plants reached only about 90% of the wild-type control. The delay in flower development may be expected to lead to a corresponding shift in the onset of senescence. The beginning of senescence was determined by first measuring the fresh weight of plants and second by quantifying the chlorophyll content of leaves during plant growth. The fresh weight of *sco1* mutant plants increased slowly and reached its peak after 7 weeks (Figure 5D). Wild-type plants reached their highest fresh weight already during the sixth week and began to decline in fresh weight during the seventh week. At its peak the fresh weight of wild-type plants was almost twice as high as that of mutant plants (Figure 5D).

Changes in chlorophyll content during leaf development and leaf senescence were measured by harvesting the fifth rosette leaf and analyzing its pigment content by HPLC. During the third and fourth week the *sco1* mutant plants still increased their chlorophyll content, whereas in wild-type leaves the chlorophyll content had already reached its maximum (Figure 5C). The chlorophyll contents of both wild type and *sco1* mutant began to decrease in the fifth week. At the seventh week the chlorophyll contents of wild-type leaves were below the level of detection but in mutant leaves small amounts of chlorophyll were still detectable (Figure 5C).

Seed set

The reduced fresh weight of mutant plants throughout their life cycle could cause a lowering of their overall vitality. The vitality of mutant and wild-type plants was compared by determining the seed production per plant. The weight of total seeds harvested from the *sco1* mutant reached with 0.11 ± 0.04 g/plant only one third of the weight of seeds produced by wild-type plants (0.3 ± 0.07 g/plant). Since the specific weight of seeds based on measuring the weights of three independent samples of 100 seeds of mutant and wild-type were the same, the amount of seeds produced in *sco1* mutant plants was severely reduced and reached only about 35–40% of that of wild-type plants.

Discussion

In the *sco1* mutant the AtSCO1/cpEF-G gene of *Arabidopsis thaliana* has been modified by a point mutation that leads to an amino acid exchange within a predicted chloroplast-localized elongation factor G. This plastid EF-G displays a 59% homology to prokaryotic EF-G proteins. The prokaryotic EF-G, a multidomain GTPase, catalyzes the translocation of the ribosome from the A to the P site on an mRNA transcript (Sharer *et al.*, 1999; Mohr *et al.*, 2000). Mutational analysis of the elongation factor G from *E. coli* revealed diverse functions of different domains during binding to the ribosomal complex and the translocation step (Borowski *et al.*, 1996; Martemyanov and Gudkov, 1999; Sharer *et al.*, 1999; Mohr *et al.*, 2000). The conserved region adjacent to the GTP-binding effector domain has been shown to be required for the interaction with the 70S ribosome (Sharer *et al.*, 1999). In the *sco1* mutant the mutation results in a replacement of the conserved glycine in position 132 by an arginine residue within this 70S-ribosome-binding region. The mRNA levels in chloroplasts of the mutant seedlings were the same as in wild-type plants, but protein levels in chloroplasts of mutant and wild-type seedlings differed greatly especially in the early seedling stage. Thus, it seems highly likely that the mutation of the EF-G protein weakens its binding to the ribosomal complex. The SCO1 protein is the only predicted EF-G protein in *Arabidopsis*. Since the *sco1* mutation is not lethal the mutated protein seems to retain a residual activity sufficient to support a basic level of protein synthesis. In the mutant, chloroplast development within cotyledons but not in true leaves is severely impaired giving rise to the formation of white cotyledons. One explanation for the mutants' phenotype could be that during the light-dependent change from heterotrophic to autotrophic growth a very high level of protein synthesis is needed to ensure the rapid transformation of etioplasts to chloroplasts. This transformation includes the

massive assembly of photosynthetic membranes and the proper insertion of large amounts of pigments that potentially may act as photosensitizers and in the light may cause major oxidative damage (op den Camp *et al*., 2003). The pigment deficiency of cotyledons may be one way of how plants avoid negative consequences of such a developmental defect. Alternatively, other cryptic plastid-specific elongation factors may exist that are expressed later during plant development.

In 3-day-old mutant seedlings the transcript concentration of some nuclear genes such as *LHCP* and *PORB* were drastically reduced. The transcription of these genes is known to be controlled by the developmental stage of the plastid compartment (Ishizaki *et al*. 2005). The mutation in the AtSCO1/cpEF-G protein seems to impact not only the translation of plastid-specific transcripts but also the retrograde control of nuclear gene expression (Surpin *et al*., 2002) and in this way further enhances the initial delay of chloroplast formation during early seedling development of the mutant.

In chloroplasts most genes of the plastid genome encode proteins that are either involved in photosynthesis or form part of the gene expression machinery (Ahlert *et al*., 2003). During plant development genes necessary for the functioning of the plastid genetic system are expressed prior to genes of the photosynthetic apparatus (Harrak *et al*., 1995; Bisanz *et al*., 2003;). Previous studies in peas have shown that chloroplast EF-G is induced early during the biogenesis of chloroplasts, and that its activity is increased during illumination (Akkaya and Breitenberger, 1992). The light-dependent transformation of proplastids to chloroplasts is accompanied by dramatic changes in the expression of genes encoding chloroplast proteins. These light-dependent changes are coordinated and controlled by different photoreceptors such as phytochromes, cryptochromes, and phototropins (Huq *et al*., 2004; Monte *et al*., 2004). Analyses of the phytochrome interacting factors (PIF) revealed that phytochromes (phy) in their Pfr or Pr form activated or deactivated the transcriptional activity of the PIF proteins. The activity of PIF1 is drastically reduced upon illumination after binding to the Pfr form of phytochrome A or B leading to a partial release of the suppressive effect of PIF1 on chlorophyll biosynthesis (Huq *et al*., 2004). In contrast, PIF3 stimulates the early light-induced expression of nuclear genes and indirectly also the expression of plastid genes. Mutation of the PIF3 gene leads to a delay in greening of seedlings (Monte *et al*., 2004).

Other proteins that are needed for regulating early steps of chloroplast formation include the nuclear-encoded plastid RNA polymerases (NEP) and sigma factors that interact and regulate the plastid-encoded RNA polymerase (PEP). The NEP is responsible for the transcription of chloroplast genes encoding proteins of the translation apparatus. Loss of the NEP, RpoT;2, causes a delay in the greening, reduction of growth, and a delay in the onset of flowering (Baba *et al*., 2004). Seedlings of the antisense lines of sigma factor 2, *sig2*, had pale cotyledons but normal green true leaves, although the expression of SIG2 protein is not restricted to cotyledons but occurs also in true leaves (Privat *et al*., 2003). A mutation in another sigma factor, *sig6*, leads also to chlorophyll deficient cotyledons and a delay in the greening of young seedlings (Ishizaki *et al*., 2005). In all these mutants the impairment of genes involved in the transcription of nuclear and plastid genes interfered with the normal development of seedlings and often caused a delay in chlorophyll accumulation mainly in cotyledons. Also mutants with a defect in the translation of plastid-specific mRNAs showed a pale or white cotyledon phenotype although the mature plants were affected, too. Seedlings of the *Arabidopsis* mutant *white cotyledon* (*wco*) develop white cotyledons and normal looking green true leaves. This defect in seedling development has been attributed to a disturbance of the maturation of the 16S rRNA in plastids (Yamamoto *et al*., 2000). Also in the *dal1–2* mutant maturation of 16S rRNA seems to be affected (Bisanz *et al*., 2003). Although in dark-grown seedlings of the *dal1–2* mutant etioplast formation occurs as in wild-type plants the subsequent formation of chloroplasts in illuminated seedlings is blocked. A similar defect in 16S rRNA maturation has been described also in the maize mutant *hcf7* (Barkan, 1993). In none of the three mutants has the mutated gene been identified and it has been concluded that the block in 16S rRNA maturation may be a secondary effect of the mutational event (Bisanz *et al*., 2003).

Conclusion

In a screen designed for the identification of mutants affected during the transition from heterotrophic to autotrophic growth of seedlings, several mutants with pale cotyledons and green true leaves were isolated. One of these mutants, *sco1*, had a defect in the chloroplast elongation factor G. This impairment seems to affect the chloroplast mRNA translation that does not only influence chlorophyll accumulation during chloroplast formation in cotyledons, but also other developmental processes such as germination and flowering. Furthermore, the vitality of the *sco1* mutant was reduced as indicated by a lower biomass production and seed set. The life-time of the mutant, however, did not change when compared with wild-type plants. Thus, in *sco1* the delay in development is not connected to life-time expectancy.

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